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Project Report: Biosignatures and Abiosignatures

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Project Progress

1. Preservation of Molecular Biosignatures

Rare samples from the Upper Oligocene Enspel Formation in Germany have previously been shown to contain exceptionally well−preserved bacterial fossils. These bacterial fossils are associated with macrofossils such as fish and tadpoles, where they replaced the soft tissues of those organisms while degrading the original organic matter. In some cases, the bacterial biofilms are sufficiently substantial that milligram quantities can be separated from the rest of the fossils and the sedimentary matrix. This distribution implies that molecular biomarkers can be studied in direct association with the morphological fossils, allowing insights into quasi−*in situ* biomarker formation. The fossil biofilms contain up approximately 50% total organic carbon and approximately 7% nitrogen. Initial studies by Co−I Steele and Postdoctoral Fellow Jan Toporski using time−of−flight−secondary ion mass spectroscopy (ToF−SIMS) have suggested that molecular biomarkers are directly associated with the bacterial fossils. To test this observation we conducted standard gas chromatography−mass spectroscopy (GC−MS) experiments on a sample where sufficient biofilms material could be separated from the sediment. For comparison, a sample from the sedimentary matrix was also analyzed. The materials were extracted using dichloromethane and methanol mixtures assisted by sonication. The extracts where then separated into six different fractions (e.g., aliphatic, aromatic, ketone, alcohol, organic acids) by silica column separation using organic solvents of different polarities and prepared for GC−MS analyses.

Preliminary data analysis of the first fraction, the aliphatic fraction, shows that there are distinct compositional differences between the biofilms and the sediment. This inference is based on three observations: the distribution of the n−alkane fraction, the presence of branched alkanes in the biofilms that are not present in the sediment, and the composition of the hopane/hopene fraction of both samples. ToF−SIMS investigation of the distribution of the biomolecules in each of the extracted samples is continuing.

Laboratory investigations of the experimental silicification and fossilization of microorganisms and viruses are being carried out in parallel. Microorganisms chosen for these experiments include *Escherichia coli* strain K–12, *Bacillus subtilis*, and *Pseudomonas putida* MnB1, as well as the filamentous bacteriophage M13. The purposes of this work are to explore potential biomarkers remnant from the fossilization process and to follow the degradation of biomarkers over time. A thorough understanding of the degradation of biomarkers over time can yield important information about what to look for in the ancient geological record, and potentially on other Solar System bodies. To date, these studies have focused on morphological studies of microorganism degradation over time (Figure 1), degradation of DNA over time with exposure to a silicifying or fossilizing solution, microbial viability with exposure to a silicifying or fossilizing solution, biomineral formation, RNA degradation over time, and changes in protein expression over time with exposure to a silicifying or fossilizing solution.

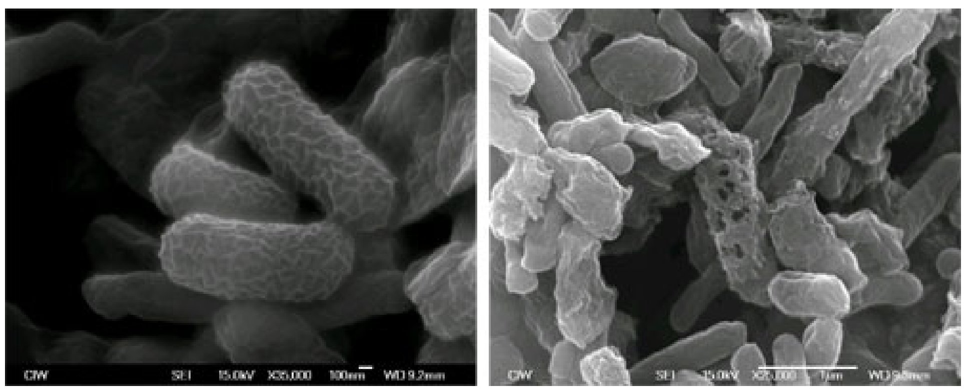

Figure 1. Scanning electron microscope images of *E. Coli* display morphological degradation during experimental silicification. The image to the left is of *E. Coli* after 3 days of exposure to 3000 ppm SiO_2. The image on the right is of *E. Coli* after 31 days of exposure to 3000 ppm SiO_2 . This contrast highlights the considerable morphological changes to the cells that occur during exposure to Si. SEM images by Rachel Schelble.

2. Stable Isotope Biosignatures

The stable C, N, O, and H isotopic compositions of a wide variety of rocks, organic materials, and living organisms are being systematically analyzed to establish the temporal and global variation in biologically–produced isotopic signatures in order to establish statistical criteria distinguishing biological from abiological signatures.

Co–I Fogel and her group have analyzed the stable C, N, O, and H isotopic compositions of a large number of Precambrian rock samples. Among these are ancient black shales, quartzites, and cherts from early Precambrian localities in western Australia sampled by collaborator John Lindsay of NASA Johnson Space Center. Postdoctoral Fellow Andrey Bekker has been conducting organic carbon analyses of Archean carbonates collected from Siberia that contain unusually heavy C isotopic ratios of +28‰. For these analyses the group developed a modified method to dissolve bulk carbonate with ultra–clean acid directly in a silver sample analysis tube. Postdoctoral

Fellow Shuhei Ono concentrated on the isotopic analysis of early Precambrian charts and shales obtained during a field trip to the Barberton formation in South Africa. Princeton University graduate student Bianca Mislowack analyzed the N abundances and isotopic content of deep subsurface rocks collected as part of a larger NAI project in collaboration with her advisor, Tullis Onstott, a member of the Indiana NAI team. These rocks contain extremely low N contents, raising critical questions as to how they sustain deep crustal microbial life. Co−I Hazen has been analyzing a suite of Precambrian stromatolite samples for carbon content and isotopic composition to determine whether the carbon is biogenic. The product of all of these efforts will ultimately be a substantial database of early Precambrian isotopic biosignatures.

In collaboration with Hans Amundson of University of Oslo, Fogel and Steele are exploring biosignatures in volcanic rocks recently erupted on Svalbard, a potential Mars analog site. Organic carbon has been discovered in vacules contained within igneous rocks. This carbon has δ^{13}C values of –12 ‰; it is thus isotopically heavier than other local sources of organic carbon, e.g., surface lichen or microbes. It is currently believed that this organic carbon must be abiologically derived. The C and O isotopic values of co−existing carbonates in both large veins and within microscopic globules indicate that these carbonates must have precipitated from glacial meltwater. A second field trip to Svalbard is planned for the coming summer. In related work carried out with Postdoctoral Fellow Marc Fries, Fogel has analyzed the isotopic composition of Antarctic meteorites. This pilot study is intended to provide background material for a larger effort to study the signatures of terrestrial contamination of meteorites that occurs during their time in and on Antarctic ice.

Co−Is Scott and Fogel have been using compound−specific isotope ratio mass spectrometric analysis of amino acids to establish isotopic signatures of discrete metabolic pathways (Figure 2). This work has been extended to studying the isotopic signatures of amino acids derived from hydrothermal vent microorganisms and abiotically synthesized amino acids produced by Miller−Urey experiments. Co−I Scott has been analyzing hypolithic microbial communities from the Mojave Desert to determine whether there are unique biosignatures associated with life in extreme dry and hot environments. Postdoctoral Fellows Jennifer Eigenbrode and Shuhei Ono initiated experiments with Fe−oxidizing microbes cultured in Winogradsky columns in anaerobic chambers with a focus on simulating the atmospheric and surface chemical characteristics of the Hadean Earth. Carbon, nitrogen, oxygen, and sulfur isotopic biosignatures will be explored as functions of time and simulated environmental conditions. Postdoctoral Fellow Albert Colman initiated studies of phosphatic minerals from the Lost City hydrothermal vent field to determine whether there is evidence of an oxygen isotope biosignature in these minerals.

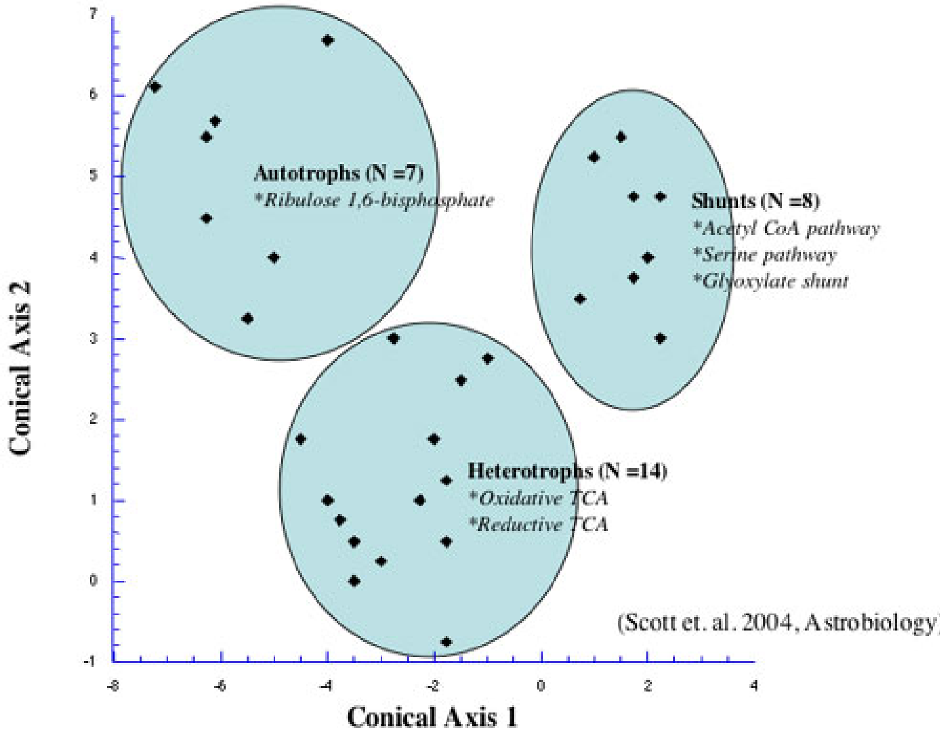

Figure 2. The δ^{13}C abundances of individual amino acids reveal functional relationships between different biosynthetic pathways utilized by prokaryotic organisms. When analyzed via discriminant function analysis, various discrete metabolic pathways can be distinguished in a two−dimensional variance representation of the correlation distances among eleven different amino acids. The conical axis 2 is dominated by the variance of the δ^{13}C of alanine with the δ^{13}C of amino acids that come directly from the TriCarboxylic Acid cycle (TCA) cycle. Conical Axis 1 is dominated by the relationship between the δ^{13}C concentrations of alanine and aspartic acid, where the degree of variance reflects differences in how the biochemical intermediates oxaloacetate, pyruvate, and alpha ketoglutarate are replenished for use by biosynthetic pathways.

3. Studies of Organic Compounds and Silicified Microbial Remains Associated with the Kamchatka Hydrothermal Region

A field trip to the silicified hot springs on the Kamchatka peninsula is planned for the late summer of 2004. The results from this field trip will be reported in the next progress report.

4. Scanning Transmission X−ray Microscopy and in situ Chemical Analysis of Organic Fossils

In March 2004 co−I Cody and colleagues traveled to the Advanced Light Source to learn how to use the scanning transmission X−ray microscope at beam−line 5.3.2. This is a state−of−the−art facility that allows for fine−scale (20−nm) spatial imaging utilizing soft X−ray absorption fine structure on the carbon, nitrogen, and oxygen K (1s) absorption edges. The group focused on the analysis of meteoritic organic matter, but in experiments planned for this fall they will focus on sub−micron−scale chemical analysis of ancient fossils. The materials to be targeted involve relatively young fossils from the Enspel Formation in collaboration with Steele and Toporski, ancient fossils derived from Precambrian cherts in collaboration with Ono and Bekker, and Cambrian materials in collaboration with collaborators Andrew Knoll (Harvard University) and Kevin Boyce (University of Chicago).

Highlights

- Molecular analysis of biofilms from the Enspel Formation reveals that within the aliphatic fractions there are distinct compositional differences between the biofilms and the sediment.
- A substantial database of C, N, O, and H isotopic biosignatures from the early Cambrian is being developed.
- Compound–specific isotope ratio mass spectrometric analysis of amino acids suggests that discrete metabolic pathways may have distinct isotopic signatures.

Roadmap Objectives

- ***Objective No. 3.2:*** Origins and evolution of functional biomolecules
- ***Objective No. 4.1:*** Earth's early biosphere
- ***Objective No. 5.2:*** Co–evolution of microbial communities
- ***Objective No. 5.3:*** Biochemical adaptation to extreme environments
- ***Objective No. 6.1:*** Environmental changes and the cycling of elements by the biota, communities, and ecosystems
- ***Objective No. 7.1:*** Biosignatures to be sought in Solar System materials
- ***Objective No. 7.2:*** Biosignatures to be sought in nearby planetary systems

Cross Team Collaborations

Co–I Fogel collaborates with John Lindsay (former NAI team at JSC); Bianca Mislowack, Princeton University (Indiana University NAI team); Hans Amundson (potential NAI international partner); and Pamela Conrad (former NAI team at JPL)

Co–I Cody collaborates with William Schopf and Andy Czaja of the UCLA NAI team.